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MEMS Independent Study: how to increase the rate of creating elastin

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This semester, my independent study was a continuation of a graduate student's, Niyousha Karbasion's, research. Her main focus was studying how to increase the rate of creating elastin, a protein found in the aortic wall. Studying elastin is essential as it provides resilience in elastic fibers and helps to shape and form tissue to manage stresses [1]. Elastin is made from a water-soluble protein called tropoelastin. When tropoelastin is produced inside the cell, it moves to the surface of the cell by binding to the elastin binding protein (EBP) [2]. Once the tropoelastin reaches the cell surface, it goes through a process called coacervation in order to make a new structure. A rise in temperature causes the tropoelastin to coacervate, and this process can be observed experimentally since the solution becomes cloudier; the elastin protein separates from the solution as it is water insoluble. If the temperature remains constant after elastin formation, a precipitate can be observed. After the coacervation process, microfibril structures are formed. Cross-linking turns the microfibril structures to elastic fibers, and they are made up of covalent bonds which increases the longevity of the elastic fibers. [2] Since the formation of elastin is important for providing elasticity to the aorta, it is essential to determine if specific substances can increase the rate of coacervation. For example, tannic acid was studied to see if it increased the rate of coacervation; it did speed up the process but could not be used on human beings since it is toxic to human cells. Pentagalloylglucose (PGG), which is derived from tannic acid, is not as toxic to human cells and seems to produce more insoluble elastin than without treatment. Therefore, the main hypothesis is that PGG can increase the efficiency of coacervation [2].

In order to test the hypothesis, various solutions of elastin can be treated with various concentrations of PGG to see if it increases the coacervation process. A microplate reader can be used to determine the rate of coacervation, as it continuously shines a light on the sample to determine the rate of absorption at a particular wavelength. Since the production of elastin causes an increase in turbidity of the solution, the absorbance should decrease with more elastin

formation. In the experiments I conducted, the elastin used was ES12. Additionally, a PGG stock solution was created and diluted by 1x PBS stock to be tested with the ES12 solution.

In the very first experiment I conducted, I repeated one of Niyousha's experiments where she tested various concentrations of a PGG solution with ES12 at 37°C. First, a 10µM solution of ES12 was created by dissolving the powder in deionized water. Next, a 100 µg/mL solution of PGG was made by dissolving the PGG powder in Dimethyl sulfoxide (DMSO). The PGG solution was diluted to various concentrations of 75 µg/mL, 50 µg/mL and 25 µg/mL using 1x PBS. The microplate reader was programmed to a temperature of 37°C, and an absorbance wavelength of 440nm. In addition, the microplate reader was set to take readings every minute for two hours. Afterwards, 100 µL of the ES12 solution and the various PGG solutions were loaded into the 96 well plate. There was also a well just containing ES12 to serve as the control, and another two wells consisting of the 1x PBS to serve as an additional control. Since the PBS does not precipitate with a change in temperature, its absorbance should remain constant. The solutions were kept on ice in order to resist a change in temperature, which causes the coacervation process to begin. Once the wells were fully loaded, they were placed into the microplate reader. Since the microplate reader program can export the data readings, it was exported and collected using an Excel data sheet. The change in absorbance as a function of time can be seen in Figure 1 below:

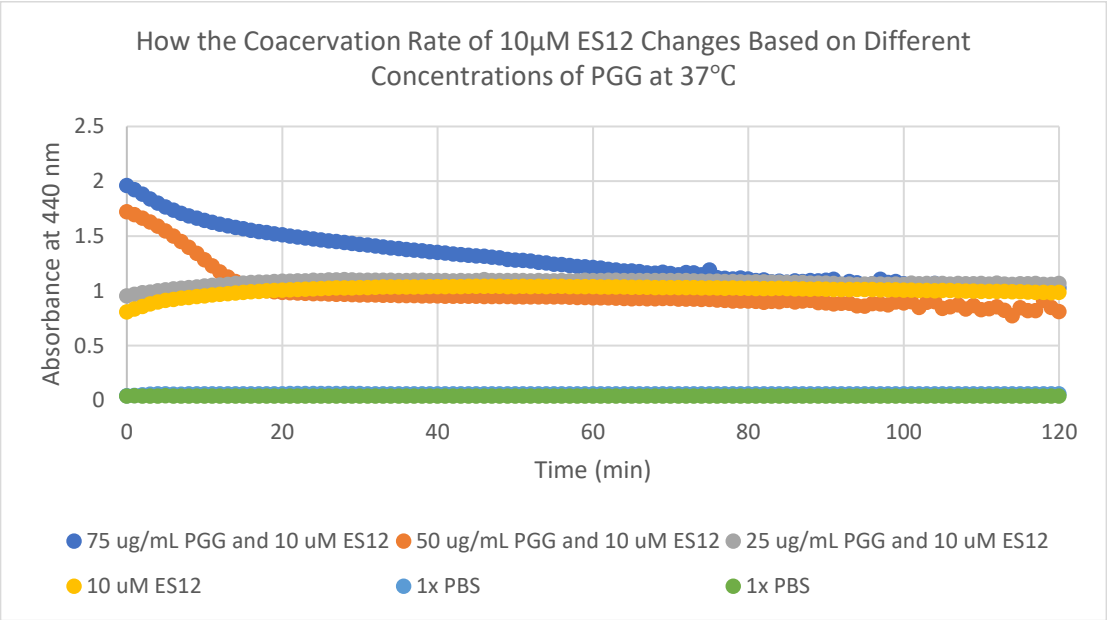

Figure 1: How the Coacervation Rate of 10µM ES12 Changes Based on Different Concentration of PGG at 37°C.

Based on the results of Figure 1, it seems that as the concentration of PGG increases, the rate of coacervation, as measured by increased absorption, increases as well. If the rate of absorbance increases with more PGG, that means that the elastin production increases as well. It also is interesting to note that the 75 µg/mL and 50 µg/mL PGG and ES12 solutions seemed to coacervate at a much faster rate than the 25 µg/mL solution; perhaps more experimentation in that range could provide insightful results such as a threshold value for coacervation. In addition, as seen in Fig. 1, the coacervation process seems to have begun before the plate was put into the microplate reader. This error can be due to not transferring the plate quickly enough to the microplate reader, and perhaps not enough ice. After observing that the ES12 and PGG solution seemed to coacervate at room temperature, I thought it would be interesting to determine the rate of coacervation at room temperature instead of 37°C.

In the next experiment, the same 10 µM solution for ES12 and the same 100 µg/mL PGG solution as used in the first experiment were created. Again, the PGG solution was diluted to various concentrations of 75 µg/mL, 50 µg/mL and 25 µg/mL using 1x PBS. Instead of programming the microplate reader to read at a temperature of 37°C, it was set to room temperature, which was estimated at 25°C. The reader also was set at the same absorbance of 400 nm and scanned the samples for two hours taking readings every minute. Once again, some wells of a 96-well plate were loaded with the 10µM ES12 and the diluted PGG solutions, and the others contained just the ES12 or the 1x PBS. The change in absorbance as a function of time can be seen in Figure 2:

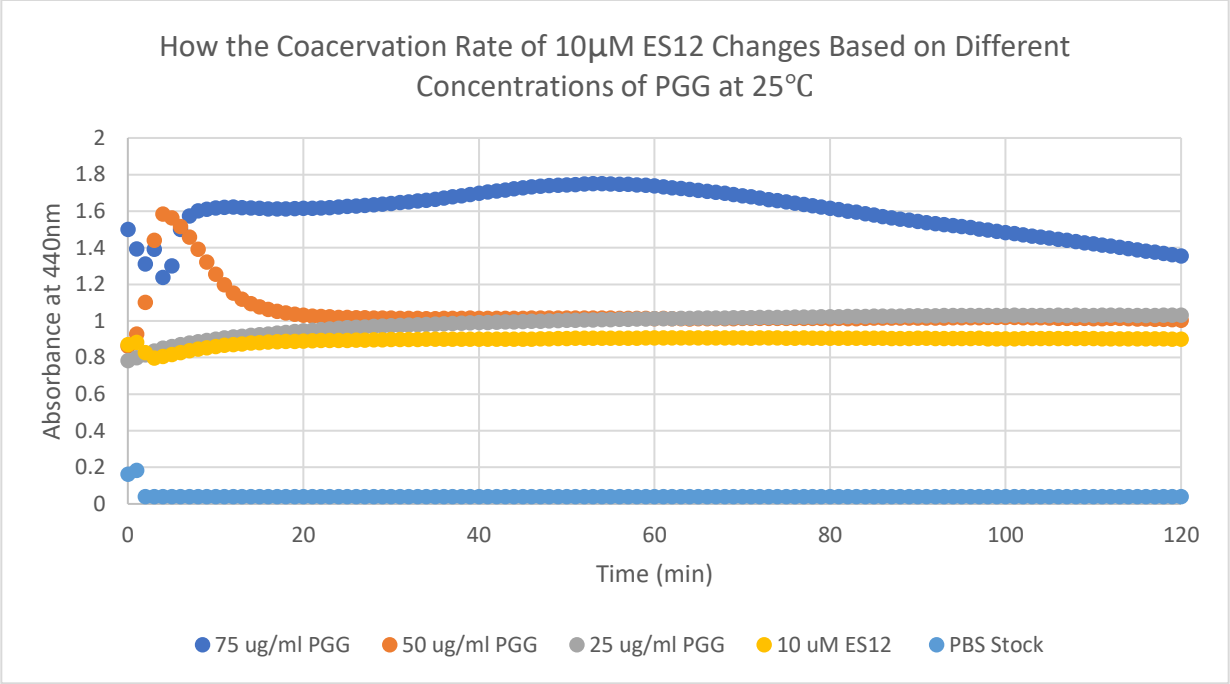

Figure 2: How the Rate of Coacervation of 10µM ES12 Changes Based on Different Concentrations of PGG at Room Temperature (25℃)

Looking at Fig. 2, it seems evident that adding a higher concentration of PGG does speed up the rate of absorption and, therefore, speeds up the rate of coacervation. Additionally, it seems that coacervation can be observed at room temperature, as the solutions with the PGG coacervated at a rate faster than just the 10µM ES12. The 75 µg/mL solution of PGG seemed to coacervate before putting the plate into the microplate reader; the curve immediately descends in comparison to the other curves that ascend and then descend. Additionally, since the curve does not descend, perhaps the lower temperature did not allow the PGG solution to un-coacervate. From the results of Experiment 1 and Experiment 2, it seems that the 75 µg/mL solution coacervates too quickly to determine meaningful experimental results. Therefore, testing at smaller concentrations, perhaps under 50 µg/mL seemed more effective. It is also important to note that the PGG solutions of 50 µg/mL and 25 µg/mL seem to return to about the same absorbance as the 10 µM ES12 solution. However, the properties of elastin may be different in these different cases.

The next experiment was conducted in order to determine absorbance values over a tighter range of numbers. Once again, the same 10µM solution for ES12 and the same 100 µg/mL PGG solution as used in the first experiment were created. The PGG solution was diluted

to concentrations of 50 µg/mL, 40 µg/mL and 30 µg/mL, and 20 µg/mL using 1x PBS. The microplate reader was programmed to a temperature of 25℃, and an absorbance wavelength of 440nm. In addition, the microplate reader was set to take readings every minute for two hours. Specific wells of the 96 well plate were loaded with and 100 µL of the 10µM ES12, and 100 µL of the different concentrations of PGG. The other wells either contained the 10µM ES12 or the 1x PBS. Below, in Figure 3 are the experimental results for the third experiment:

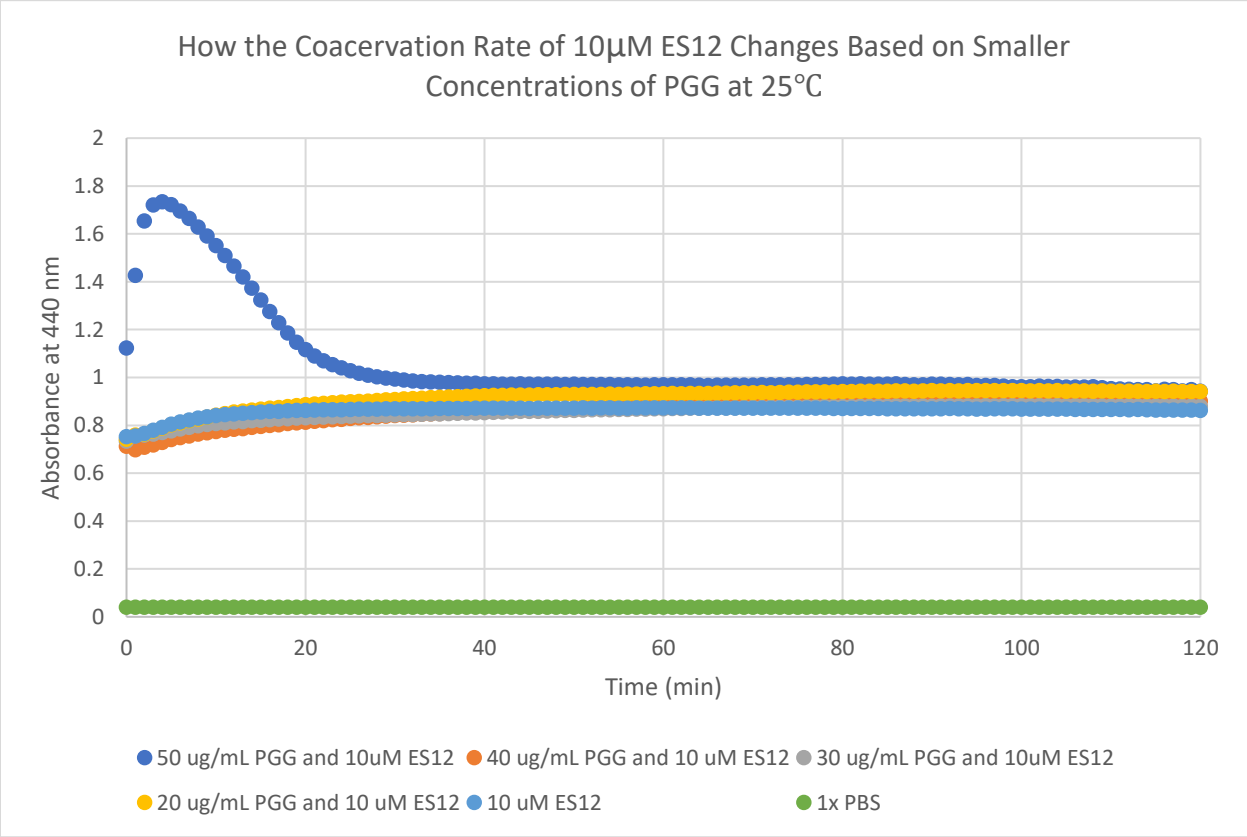

Figure 3: How the Coacervation Rate of 10µM ES12 Changes Based on Smaller Concentrations of PGG at Room Temperature (25℃).

As seen in the above figure, there seems to be a stark difference in the rate of absorption for the 50 µg/mL PGG and 10 µM ES12 solution; the rest of the of the curves containing ES12 seem to have around the same rate of absorption. Since the concentrations were relatively close in value, this result was not expected. Perhaps the results were due to the DMSO and PGG solution not being in the fridge for a long enough time before conducting the experiment. Coacervation is very temperature sensitive, therefore, a slight change in temperature for the PGG solution could have caused the coacervation process to be affected. This led me to believe that perhaps there could be a threshold for the amount of PGG required for a noticeable difference in the rate of

absorption. However, before making this conclusion, this experiment was conducted once again to see if there maybe was a threshold value for the coacervation of ES12 and PGG. The same procedure was followed, and the results for this experiment can be seen in Figure 4:

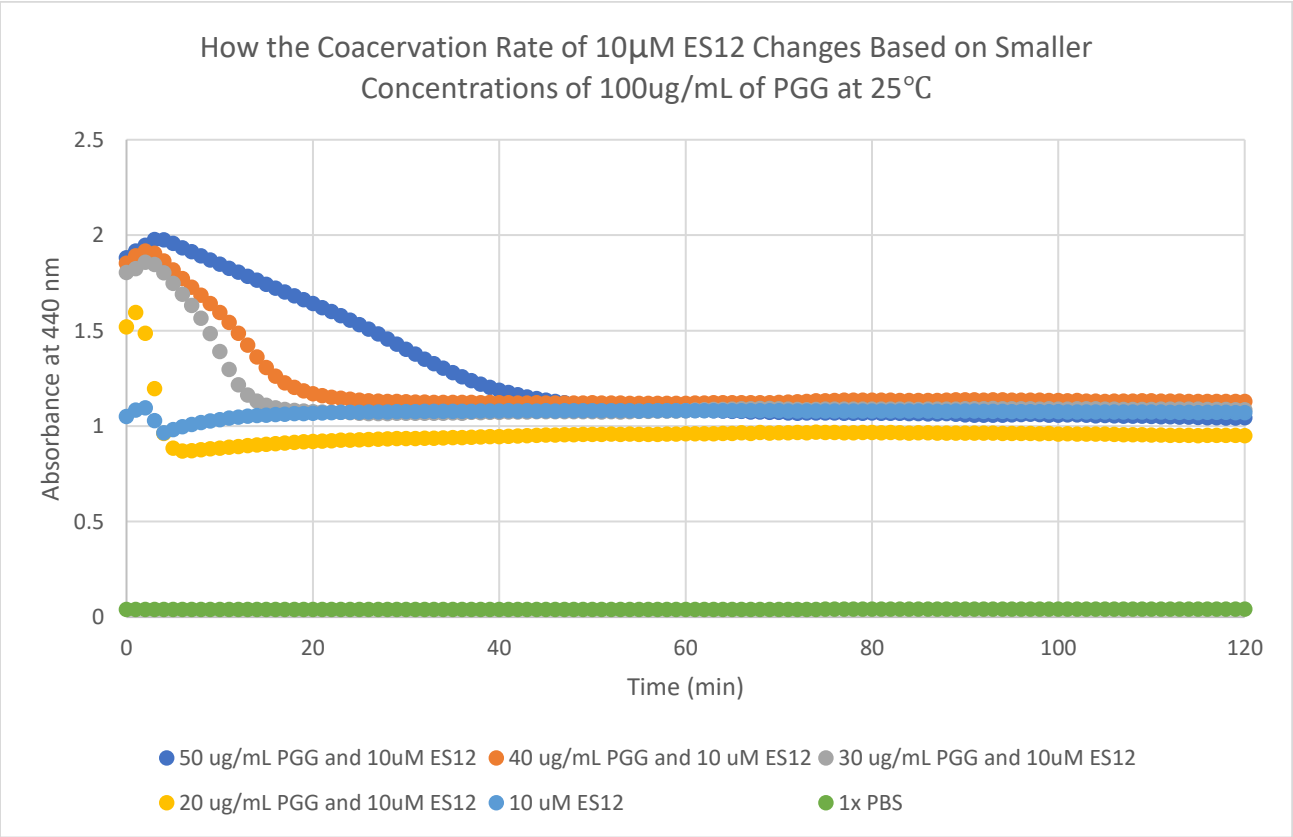

Figure 4: How the Coacervation Rate of 10µM ES12 Changes Based on Smaller Concentrations of PGG at Room Temperature (25°C) for the Second Experiment.

Looking at Fig. 4, it does seem evident that adding PGG does increase the rate of absorption, and, therefore, it seems that PGG can increase the speed of coacervation. Additionally, it seems that room temperature is a sufficient change in temperature for the coacervation process to begin. Since the concentrations were smaller for each PGG solution, the curves should be closer together in the rate of absorption. This can be seen in Fig. 3, as the curves seem to be very close to one another.

Looking at the results from the experiments, there are some sources of error that could have contributed to some of the inconsistencies between each experiment. Coacervation is a temperature sensitive process, so any small change in temperature could have contributed to the error. First, perhaps the coacervation process could have begun when the ES12 solution was

created with the deionized water. Although the solution was put onto ice immediately after dissolving the ES12 into the water, perhaps a slight change in temperature could have affected the coacervation process. In addition, the PGG and DMSO stock was recreated for the first two experiments and the first experiment in experiment three. The reason why this solution was recreated for each experiment was since it froze in the fridge when it was stored, and I was unsure if the PGG and DMSO freezing together could have affected its properties. At the end of the first experiment in experiment three, the PGG and DMSO solution was aliquoted for future use and stored in the fridge. Interestingly, as seen in Fig. 3 and Fig. 4, the absorption rate was greatly different in the two experiments in experiment three. Perhaps freezing the DMSO and PGG solution can affect the coacervation process; this is something that can be studied with future experimentation.

It is also interesting to note that the initial rate in absorption for the solutions containing higher amounts of PGG had a steeper slope than those of the solutions containing a lower amount of PGG or no PGG at all. However, as time progressed, when the absorption began to decrease, the solutions with a higher concentration of PGG seemed to have a shallower slope. Perhaps this can be due to a change in elastin properties over time and can be tested in future experimentation.

References:

[1] J. Moore and S. Thibeault, “Insights into the role of elastin in vocal fold health and disease,” Journal of voice : official journal of the Voice Foundation, May-2012. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3190022/#:~:text=FUNCTION%20OF%20ELASTIN,and%20designed%20to%20manage%20stress.&text=Elastic%20fibers%20give%20vertebrate%20tissues,quality%20for%20normal%20homeostatic%20function. [Accessed: 02-May-2021].

[2] Karbasion, N., 2020, “Fall 2020 Rotation Report”, Washington University in St. Louis, St. Louis, MO.